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THE FLUORESCENTAICROSCOPIC DESCRIPTION OF RICKETTSIA EURNETY AND THEIR PHOTOGRAPHIC REPRODUCTION

H. Urbach M. Sprossig

16 April 1965

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Following to the trunchtion of an article by M. Urbach and M. Darossin, published in Meas, Bales I. Orig. 161: 39-44, 1954. Translated by B. MacDonald.

For the first time in Serman literature H. Traberg (1) in 1946 and 1947 reporting on the microscopic destriction of Rickettsia burneti (R. burneti) and by this opening an equipleteral sphere of action, that by the study of these agricultures and the diagnostic of 1-fever is characterized with microscopic methods.

Since this time a series of works have appeared, bear in mind the morphological view point in the illustration of R. North ti with the light and electron microscope (3). As yet the process most frequently used of microscopic visualizations with the help of the light microscope avails itself of the color technique according to disma or with Victoria blue according to Herzberg. In order to preserve easily visible pictures, it is necessary to administer to the diems are a 1-3 daily preservation of freshly made preparation, in the Victoria blue dye a once daily preparation is enough.

It is nevertheless often recessary on a technical working basis, as soon as possible, after production of a preparation to make a decition about it, if the rax naterial used is an agitator producer or further treatments are necessary and so on. Especially in the traduction of antique from vitelline sac cultures from R. horneti the susstion of excitors to the complement fixation reaction it very significant, since on their reaction depends further treatment and processing of vitelline sac material to the antique. The work undences an objectionable interruption, when first a day must be writed for in order to provide a basis to decide on the dre-technical production of Rickettsia. For us the necessity appears from that has been said, after seeking a process of preparation, that interistely after production of the preparation demands a whore lesting treatment of the same and yet guarantees a good general view in the microscope investigation. The advised requisition under consideration appears appropriate to us, for the valuation the flourescent microscopy is referred to.

The founiation for the development of the flourscent microscope took place in 1904 by a. Mohler, Zoist-Jenz, by the knowledge that biological objects flouresee with exposure to monochormatic Ultraviolet (UV) rays. A. Kohler and H. Diede topf Comonstrated in 1908 in Vienna the mathod of dark field illumination with UV, this simultaneously was the first knowledge produced about fluorescent microscopes. H. Leinson, Microscopes, improved the filter, so that only UV-rays between 250 au and 400 mm penetrate the object. A carbon are law serves as a UV source. The firm G. Seichert developed a similar design in 1911, however they found from carbon area applicable to UV pro-

duction. S.V. Provessi ands in 1914 the first fluorescent scienting and S. Bedder and in 1925 colored animal anterial with fluorescent substracts (impactively). It is the especial assist of Maitingers, latter a content fluorescent accordate (Theoretica) have been reported as their weelfulner in these cuts and now color efficies, the secondary of the fluorescence. In 1937 maith: Magement see then referred to the Theoretica in the production of protozoa busturia and different virus broads.

Eno promotiple of fluoroseens microscopie decimie of a move afterward that the fluoroshrone impressed decimes a visible and invisible UV rays change to visible higher, through which the fluoroshroned substratums are observed to be call lighting bodies and with the usual optic of a microscope can be persoived. The princry or solf fluoresence in least sivalficate for medical microbiology. On the centrumy Fluoroshroned which accepts selectively as age material on special elements that be used. These phenomena are reduced to an electrockroup, tive or electrostable process. In order to until the fluoroshrone with objects described, decriented assure preparations with pluoroshromes can be protapped. Purthernore, it is also possible to headle the culture media with one. Fluoroshromes, which were taken out from the developing micro-organisms in the matricate to hast organisms fluoroscopy chemothers which is also possible action. Finally the possibility still exists, to administer to hast organisms fluoroscopy chemothers which in parasites.

For our purcoss only the fluorochroming of accomprenations is opplicable. Sold fluorochromes can cause a polychrologic flock. Their application always results in strong dilutions can ac 1:55 to 1:300000 and for these times of a few accomis or a minute. In the call of ebjects which applies not proved in their behavior opposite. Mucrochromes, it is recommed then in one event of great dilution in other to everice the direction of color time. By this means eventalism on be avoided. Whe pluorochrome solutions were a stiffer a proparation only ones. Therefore the use of the bubble is not recommended, the une can be consided in clide alerra. The proparation of the fundamental for a popular control of the fundamental and donger lasting emposure of the premate tion to the UV ray with with the fluorescent microcoops.

1. Fluorescentzioresespie Research

In our fluorescent dicroscents research we make use of lage luminescence equipment with the carbon are large of the Zuico-Jane first. The curved limbs in stratification of a cooling bulb with a 4, colution of coppen sulface, thereby the Loding we red portions are discommensed, in order to make a curico different thicker blue and filters (at 115 miles and 212 miles are thicker blue and filters (at 115 miles and 212 miles are the suitable wave length and size of the suitable wave length and size of the suitable wave length and size of the fourth miles are the filters seen to be suitable to the suitable wave and could be for the filters of the suitable wave and the suitable of the filters of the suitable wave and the suitable of the filters of the suitable of the filters of the suitable of the suitable of the suitable of the filters of the suitable of t

Ammercians objective 99/1.25 and a 5 or 10 fold-soular amplies - tion. We notally use to the Amiltonia in our research the Uvetu Filter Br Fidency, along the last illustration to obtained with it. Attill the disturbing UV repe, which pendurate the object, must be inserted to read into a interview tilt me, which are expected to the coular. A damic gold filter proved to us as very expropriate.

We prepared the preparations from antipon material, as it is obtained by up for the accomplishment of the complement fixation reaction, besides from vitalline accomplanes and in the form of testicle spot accura from infected guines pize.

After the air drying and with fination of the smear we have a complete series, which often immediately treated and were also often preserved form many days, colored withthe following fluore-chromes: Frinulin, Avramin, Audicin 3 60, nothylviolet earm, neutral red extra, brilliantdiamil green F or Fa. Dr. G. Loubler and Co., Leinzig. For the study of the strain "Grita" served us in 70 to 85 egg passages and "Schauben in 40 to 50 egg passages.

We maintain the supposition, that itself, according to the observations of P.K.H. Hasemanno (5) or virus preparations, Privalin was spontaneous at tiret for the choicetion of R. Westell However, only slightly light illustrations appear in the trimular fluorochroning. The rickstain do now poises a natural, that is spontaneous fluorochroning is necessary. Of all fluoroccurs, so that a fluorochroning is necessary. Of all fluoroccurs, considered in different spear preparations, the luminian according to our source demonstrated to us, a light vehicu soluble diphendimetram piccont in actor, as the best available. The light intensity of the rickstatic fluoroccursed with luminiar plainty oversholded the Friedlin, that is precent it described on, the mode of application of the condin is veried. The others in addition to the fluoroccurs rentioned converted to be useless for the production of r. burnata.

First we used an imparing solution of 1:500, treated the first paration 2 seconds with it and unsted it for a few records with cold water. Alchettsia were indeed produced, but the vitalling dae naterial still flocked un vallouish green, so that the contrast between rickettain and underground material aid not expedit clearly grouph. In 10 seconds after washing with approximator of about 60°C the decolorization of the recurrent with with this way show up brightly. The subsequent treatment with with which water about the course not be extended over 10-15 sections. They observe the rickett-sia or surmin could leave and undergo taming to their intensity. After changes in degree of day time (in F min.) and the concentration of imparin solution (191000) the following technique for obtaining batter production has been proved counce, which are cuitable for microscopic regradiations correction of Q.S. phenol liquorest, for 30 seconds, following damaging of the phenol liquorest, for 30 seconds, following descriptions of the slide in a glass of where water at 50°C for 1-15 seconds.

according to the thickets of the presumation, Go sicution on uses fluorescent free incorpics oil [1]:5").

If the the fluorescent place and is madicated of p. hyperticle and of the first production of the fluorescent production of the first production of the fluorescent production of the vibration of missions of the first production of missions of missions of the first production for the configuration of missions of the first production. The production for the configuration of missions fluored to the first production for the configuration of missions fluorited repetion. The first production for the configuration fluorited repetion. The first production in the configuration in the first production in the second formulation repetion, the first production in the second first production in the first production of constraints the bright production chinnin bisheutida in the production not given.

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- i). The leng composite this proving a monotone corrections and finally an analysis of allein a proving the rese and thereby by the correction vibration 1 and by measuring to a blurred picture. The chart is chested in the care of a small lens of the without actitional observation oculars and also for that reason they cannot be corrected.
- 2) In the case of a longer embourd time the radiation consistivity of the rickettoia loads to a clour diminution of its illuminating power, as also has been observed in other siero-organisms.
- 5) We have the impression, that the effect illuminating power of the church plane adoc over and beyond to the extended rickettein particles in longer empoure to the negative material and is produced as a weak copens. This characters then leads to blurred contours of the fluorescing rickettein.

Since in the case of the optical tools used by us the shorter exposure time can be possible effect by the incertion of a suitable operative of the objectives of the only curies remains in order to compensate for a ten fold characterist emposure time with a 2 hour develocing time. We support the support to this method by Mister 7. Froll and Dr. 1. Otto, Zeiss-Jena (11-12).

We found out by a series of furnher test exposures the nest suitable proportion for our test was a shorter exposure time and finer grained filt materials. We obtained the test performance with the following operation: use of Agra-F-filtes of 17/170 Dim., exposure time of 5 minutes with an idlustration criterian 900:1 (corresponds to a mercal composure of 50 minutes). Two four development of the rim with untraffuent and mater. Redow the picture on to extra hard paper and devolop with Blautal (Left).

With this method we estained on this basis the short exposure for the larger magaitude of n. https:// which shows in the fluorecent microscope on a very weak light source, a picture repreduction, as we can never obtain it of the same quality with the usual common methods even after entended trate. In their structure the larger nuclei can be produced with fine datail, which is not visible by observation with the named eye. The regular corresponding fine grey have on the negative acterial is without manning for the production of unotal positive. The resulting close gradations in the method with the mail variations in light intensity of microscopic victures is accomplished in no detrimental way. The correspond notative is necessarily for 1,2,3 and 4 must be reduced on an additional photographic supplemental magnification caused by the miruteness of the reduced. It has not been estimated as a difficiency of the adsorptive technique itself.

lifter our experience the short exposure with longer develo-

ping time produced a useful method for photographic representation in fluorescent microscopy.

SUNMARY

With the help of the fluorescent microscope the rickettsia burneti can be produced by different materials (Antigen, vitelline sac, testicle smears). Auramin 1:500 is especially suited as a fluorochrome. The microscopic fluorescent picture can be best obtained with 17/17° with short exposure time (5 minutes) and long development time for illustrations.

The med.-techn. assistant Frl. E. Hartmann-Heyn has afforded us valuable help with the making and photographic production of the preparation.

FIGURE CLARIFICATION

The figures (strain "Grita") show in the figure criterion of 900:1 and of a remagnification of 2000:1 in:
Figures 1 and 2. Smear of infected 8 day pre-incubated fowl eggs, 5-6 daily vitelline sac material.

Fig. 3 and 4. Test spot preparation of guinea pig abouton the fifth day of fever. Rickettaia near and between the histiosyt nucleus.

Fig. 5. Series of rickettsia suspensions as antigen for the complement fixation reaction.

Fig. 6. Bright field absorption of a test spot preparation. Histocyt vacualized with intracellular rickettaia in chains or thread arrangement (vacuale cell according to Herzberg). The nucleus is deformed single shaped and is pressed in the cell perifery.

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Urbach u. Sprößig, Fluorrecenmikraskopische Darstellung der Rickettsis burneti

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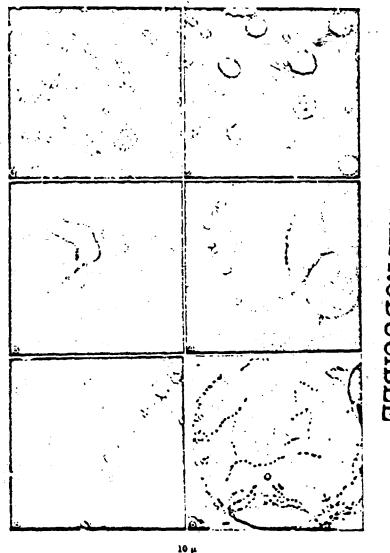
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